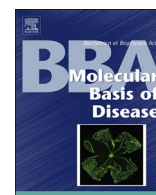


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Berberine-induced cardioprotection and Sirt3 modulation in doxorubicin-treated H9c2 cardiomyoblasts



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ABSTRACT

Doxorubicin (DOX) is one of the most widely used anti-neoplastic agents. However, treatment with DOX is associated with cumulative cardiotoxicity inducing progressive cardiomyocyte death. Sirtuin 3 (Sirt3), a mitochondrial deacetylase, regulates the activity of proteins involved in apoptosis, autophagy and metabolism. Our hypothesis is that pharmacological modulation by berberine (BER) pre-conditioning of Sirt3 protein levels decreases DOX-induced cardiotoxicity. Our results showed that DOX induces cell death in all experimental groups. Increase in Sirt3 content by transfection-mediated overexpression decreased DOX cytotoxicity, mostly by maintaining mitochondrial network integrity and reducing oxidative stress. p53 was upregulated by DOX, and appeared to be a direct target of Sirt3, suggesting that Sirt3-mediated protection against cell death could be related to this protein. BER pre-treatment increased Sirt3 and Sirt1 protein levels in the presence of DOX and inhibited DOX-induced caspase 9 and 3-like activation. Moreover, BER modulated autophagy in DOX-treated H9c2 cardiomyoblasts. Interestingly, mitochondrial biogenesis markers were upregulated in in BER/DOX-treated cells. Sirt3 over-expression contributes to decrease DOX cytotoxicity on H9c2 cardiomyoblasts, while BER can be used as a modulator of Sirtuin function and cell quality control pathways to decrease DOX toxicity.

1. Introduction

The anthracycline Doxorubicin (DOX) is one of the most prescribed and effective anti-cancer agents [1,2]. The clinical use of DOX has been associated with a cumulative and dose-specific cardiotoxicity that involves the development of congestive heart failure [3–6]. Being a very potent chemotherapeutic, a more effective prevention of DOX-induced cardiotoxicity is needed in order to potentially optimize the effective dosage given to patients and therefore reduce morbidity.

Adult cardiac progenitor cells (CPCs), present in adult heart, contain stem cell characteristics and are involved in tissue homeostasis and myocardial regeneration during pathological conditions [7–9]. CPCs have been studied in the context of several pathological conditions in animal and humans including as a pathophysiological target against DOX cardiotoxicity [9–12]. One relevant model to investigate DOX-induced effects on CPCs is the H9c2 cell line, which has morphological

characteristics similar to immature embryonic cardiomyocytes [2,13–16].

It has been previously described that DOX, at 0.5 and 1 μ M concentrations, equivalent to those achieved in the plasma upon administration of therapeutic doses [14], promotes metabolic stress and increases protein acetylation of crucial mitochondrial enzymes [17–19]. Sirtuins are NAD⁺-dependent deacetylases that catalyse the deacetylation of histone and non-histone lysine residues, also having protein ADP-Ribosyltransferase activity [20]. Sirtuin 3 (Sirt3) is the major mitochondrial deacetylase [21], modulating several mitochondrial pathways [22]. Sirt3 is involved in the maintenance of oxidative phosphorylation during stress, regulation of ROS generation at the electron transport chain (ETC), as well as detoxification of ROS via activation of antioxidant enzymes, such as glutathione and superoxide dismutase 2 (SOD2) [17,23–25]. Moreover, it was shown that Sirt3 was capable of deacetylating OPA1, contributed to the preservation of

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